

## Technical Data Sheet

## Bromodeoxyuridine (BrdU)

## Product Information

Material Number:	550891
Size:	25 mg
Storage Buffer:	Aqueous buffered solution filtered through a 0.22 µm filter

## Description

Bromo-deoxyuridine (BrdU) is a thymidine analog that is used in cell proliferation studies. BrdU in culture is incorporated into the DNA during DNA synthesis. Cellular incorporation of BrdU can be detected by anti-BrdU specific antibodies following membrane permeabilization by flow cytometry or immunohistochemistry. The molecular weight of BrdU is 307.1.

## Preparation and Storage

Store product at -80°C prior to use or for long term storage of stock solutions.

This preparation contains no preservatives, thus it should be handled under aseptic conditions.

Avoid multiple freeze-thaws of product.

Five vials are provided. Each vial contains 0.5 ml of a 10 mg/ml BrdU solution diluted in 1X DPBS.

The BrdU solution once thawed is stable at 4°C for 4 months.

## Application Notes

## Recommended Assay Procedure:

Prior to BrdU immunostaining to detect proliferating cells it is necessary to label the cells or tissue with BrdU. Labeling can either be done in vitro for cell cultures or performed in vivo for experimental animals. Both protocols are provided.

## In vitro labeling of cultured cells and cell lines with BrdU

Many different protocols for in vitro BrdU labeling of cells have been reported. We have found that incubating cells with BrdU at a final concentration of 10 µM in cell culture medium is effective for labeling a wide variety of human and mouse cell lines and normal cell populations.

To label cells in vitro, carefully add 10 µl of a 1 mM BrdU working solution (dilute BrdU Stock Solution 1:30 in tissue culture media) directly to each ml of tissue culture media. For this step, it is important to avoid disturbing the cells in any way (e.g., by centrifugation steps or temperature changes) that may disrupt their normal cell cycling patterns. The cell culture density should not exceed  $2 \times 10^6$  cells/ml. The treated cells are then incubated for the desired length of time. The incubation time with BrdU is dependent on the test cell population's rate of cell cycle entry and progression. For example, an effective length of time for pulsing an actively proliferating cell line (e.g., CTLL-2 cells) is 30-45 minutes, (i.e., when the cells are in the logarithmic phase of cell proliferation). Investigators should determine the incubation times that are optimal for each different cell line or cell population within a particular experimental system. Cells from the same population that are not BrdU-labeled are the negative cell staining control.

## In vivo labeling of mouse cells with BrdU

Two common methods used for in vivo BrdU labeling of tissues and cells include the intraperitoneal injection of a BrdU-containing solution into mice and the feeding of mice with BrdU that is added to their drinking water.

## a) Intraperitoneal Method:

A 10 mg/ml solution of BrdU in sterile 1X DPBS is provided for in vivo use. Inject mice i.p. with 100-200 µl (1-2 mg) of BrdU solution.

Incorporation of BrdU can be detected in thymus and bone marrow in as little as 1 hr post injection. 24 hrs post injection BrdU can be detected in most of the tissues.

## b) Drinking water method:

Dilute BrdU to 0.8 mg/ml in the drinking water. The BrdU mixture should be made up freshly and changed daily. Prolonged feeding of BrdU can have toxic effects. Some investigators have reported lethal effects associated with 14 days of continuous BrdU feeding. For longer term studies, some investigators have reported that feeding mice with BrdU for 9 consecutive days followed by a change over to normal water has worked effectively. BrdU incorporation by cells from these animals has been detected past 70 days.

## Suggested Companion Products

Catalog Number	Name	Size	Clone
555627	Purified Mouse Anti- BrdU	0.1 mg	3D4
560209	Alexa Fluor® 647 Mouse anti-BrdU	100 tests	3D4
563445	BV510 Mouse anti-BrdU	50 tests	3D4
554656	Stain Buffer (FBS)	500 ml	(none)

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## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.

## References

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